

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : A61K 31/557 // (A61K 31/557, 31:415, 31:27)		A1	(11) International Publication Number: WO 97/23226 (43) International Publication Date: 3 July 1997 (03.07.97)
(21) International Application Number: PCT/US96/18888 (22) International Filing Date: 25 November 1996 (25.11.96)		(81) Designated States: AU, CA, JP, MX, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).	
(30) Priority Data: 08/577,038 22 December 1995 (22.12.95) US		Published <i>With international search report.</i>	
(71) Applicant: ALCON LABORATORIES, INC. (US/US); 6201 South Freeway, Fort Worth, TX 76134-2099 (US).			
(72) Inventor: DEAN, Thomas, Robert; 101 Meadow View Court, Weatherford, TX 76087 (US).			
(74) Agents: YEAGER, Sally, S. et al.; Alcon Laboratories, Inc., Patent Dept. Q-148, 6201 South Freeway, Fort Worth, TX 76134-2099 (US).			

(54) Title: COMBINATIONS OF PROSTAGLANDINS AND MIOTICS FOR LOWERING INTRAOcular PRESSURE

(57) Abstract

Methods for controlling glaucoma or ocular hypertension with combinations of DP-agonists and miotics are disclosed.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AM	Armenia	GB	United Kingdom	MW	Malawi
AT	Austria	GE	Georgia	MX	Mexico
AU	Australia	GN	Guinea	NE	Niger
BB	Barbados	GR	Greece	NL	Netherlands
BE	Belgium	HU	Hungary	NO	Norway
BF	Burkina Faso	IE	Ireland	NZ	New Zealand
BG	Bulgaria	IT	Italy	PL	Poland
BJ	Benin	JP	Japan	PT	Portugal
BR	Brazil	KE	Kenya	RO	Romania
BY	Belarus	KG	Kyrgyzstan	RU	Russian Federation
CA	Canada	KP	Democratic People's Republic of Korea	SD	Sudan
CF	Central African Republic	KR	Republic of Korea	SE	Sweden
CG	Congo	KZ	Kazakhstan	SG	Singapore
CH	Switzerland	LI	Liechtenstein	SI	Slovenia
CI	Côte d'Ivoire	LK	Sri Lanka	SK	Slovakia
CM	Cameroon	LR	Liberia	SN	Senegal
CN	China	LT	Lithuania	SZ	Swaziland
CS	Czechoslovakia	LU	Luxembourg	TD	Chad
CZ	Czech Republic	LV	Latvia	TG	Togo
DE	Germany	MC	Monaco	TJ	Tajikistan
DK	Denmark	MD	Republic of Moldova	TT	Trinidad and Tobago
EE	Estonia	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	UG	Uganda
FI	Finland	MN	Mongolia	US	United States of America
FR	France	MR	Mauritania	UZ	Uzbekistan
GA	Gabon			VN	Viet Nam

IN THE UNITED STATES PATENT
AND TRADEMARK OFFICE

5

10

15

**COMBINATIONS OF PROSTAGLANDINS AND MIOTICS
FOR LOWERING INTRAOCULAR PRESSURE**

20 This invention is directed to methods for treating persons suffering from glaucoma or ocular hypertension.

Background of the Invention

25

Although the underlying causes of glaucoma are not well understood, its symptoms often include elevated intraocular pressure (IOP) which arises due to inadequate outflow of aqueous humor through the trabecular meshwork. Also, ocular hypertension, a condition characterized by elevated IOP, can be a risk factor in the development of glaucoma. If left untreated, or if inadequately treated, glaucoma can lead to blindness or significant vision loss. Therefore, there is a continuing need for therapies which control elevated IOP associated with glaucoma or ocular hypertension.

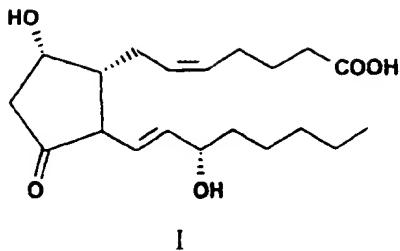
There are currently a number of drugs used to treat persons with glaucoma, including: miotics (e.g., pilocarpine, carbachol, and acetylcholinesterase inhibitors); sympathomimetics (e.g., epinephrine and dipivalylepinephrine); alpha-2 agonists (e.g., para-amino clonidine); beta-blockers (e.g., betaxolol, levobunolol, and timolol); and carbonic anhydrase inhibitors (e.g., acetazolamide, methazolamide, and ethoxzolamide systemically and dorzolamide topically). Miotics and sympathomimetics are believed to lower IOP by increasing the outflow of aqueous humor through the trabecular meshwork, while beta-blockers, alpha-2 agonists, and carbonic anhydrase inhibitors are believed to lower IOP by decreasing the formation of aqueous humor.

10

In addition, although none have yet been approved for anti-glaucoma therapy, certain classes of prostaglandins and prostaglandin analogues (hereinafter collectively referred to as "prostaglandins") have been shown in various animal models and in some clinical studies to reduce IOP to a greater extent than most currently used therapeutic agents. See, for example: U.S. Patent Nos. 4,097,489; 4,599,353; 5,296,504; WO 90/02553; EP 569 046A1; EP 364 417B1; U.S. Patent No. 4,994,274; and EP 289 349. In contrast to the case with miotics which affect conventional outflow, i.e., via the trabecular meshwork, some prostaglandins are believed to lower IOP by increasing the outflow of aqueous humor via the uveo-scleral route.

15
20

Prostaglandins are metabolite derivatives of arachidonic acid. The arachidonic acid cascade is initiated by the conversion of arachidonic acid to prostaglandin G₂ and subsequent conversion to prostaglandin H₂. Other naturally occurring prostaglandins are derivatives of prostaglandin H₂. A number of different types of prostaglandins have been discovered including A, B, D, E, F and I-Series prostaglandins. Of interest in the present invention are compounds which exhibit similar IOP lowering mechanisms as PGD₂ with the following formula:



The relationship of PGD₂ receptor activation and IOP lowering effects is not well known. Various publications have reported that PGD₂ receptor activation leads to second messenger activation and in particular, to the stimulation of adenylate cyclase and resultant increases in cAMP levels (Thierauch, "Prostaglandins and their Receptors: II. Receptor Structure and Signal Transduction," *Journal of Hypertension*, 12:1-5 (1994)). Regardless of mechanism, PGD₂ has been shown to lower IOP (Nakajima, "Effects of Prostaglandin D₂ and its analogue, BW245C, on Intraocular Pressure in Humans," *Graefe's Archive Ophthalmology*, 229:411-413 (1991)).

Synthetic PGD₂-type analogs have been pursued in the art (*Graefe's Archive Ophthalmology*, 229:411-413 (1991)). Though PGD₂-type molecules help lower IOP, these types of molecules have also been associated with undesirable side effects resulting from topical ophthalmic dosing. Such effects include an initial increase in IOP, conjunctival hyperemia, increases in microvascular permeability, and increases in eosinophile infiltration (Alm, "The Potential of Prostaglandin Derivatives in Glaucoma Therapy," *Current Opinion in Ophthalmology*, 4(11):44-50 (1993)).

20

It has been unexpectedly found that 3-Oxa-D-prostaglandins ("3-O-DP") are more efficacious in lowering and controlling IOP than their 3-carba analogs. Consequently, these 3-O-DPs can be administered at lower doses than their 3-carba analogs. The lower doses are believed to reduce their activity on other prostaglandin receptors and thereby reduce many of the aforementioned undesirable side effects.

All six types of therapeutic agents discussed above have potentially serious side effects. Miotics, such as pilocarpine, can cause ocular discomfort, headaches, and blurred vision, which may lead either to decreased patient compliance or to termination of therapy. Systemically dosed carbonic anhydrase inhibitors can also cause serious side effects, such as, 5 nausea, dyspepsia, fatigue, and metabolic acidosis, which affect patient compliance and/or necessitate the withdrawal of treatment. Some beta-blockers have increasingly become associated with serious pulmonary side effects attributable to their effect on beta-2 receptors in pulmonary tissue. Prostaglandins often produce hyperemia and edema of the conjunctiva.

10 Patients with extremely high IOP require a therapy regimen which includes the use of two or more pharmaceutical compositions containing drugs selected from two or more of the above-cited classes. There is a continuing need for new, more potent anti-glaucoma therapies. The present invention is directed to such therapies.

15 **Summary of the Invention**

The present invention is directed to the use of DP-agonists (hereinafter defined) and miotics, particularly pilocarpine, for treating persons suffering from glaucoma or ocular hypertension.

20

Description of Preferred Embodiments

Many people suffering from glaucoma or ocular hypertension cannot control their elevated IOP using only one of the therapeutic agents described above. It is believed that the 25 use of a miotic, such as pilocarpine or an analogue of pilocarpine (hereinafter collectively referred to as "pilocarpine"), in combination with a DP-agonist will be effective in controlling the IOP in those persons for which one therapeutic agent is not enough.

As previously discussed miotics are known to lower IOP by increasing outflow via the trabecular meshwork. DP-agonists may affect IOP by decreasing aqueous humor production (decreasing inflow). Thus, the use of a miotic, such as pilocarpine, carbachol, or acetylcholinesterase inhibitors, preferably pilocarpine, in combination with a prostaglandin that decreases aqueous humor production will better lower and control elevated IOP in persons suffering from ocular hypertension or glaucoma.

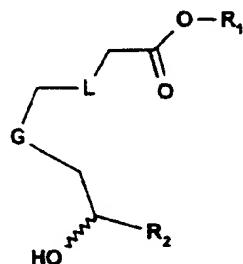
The DP-agonists of the present invention are useful in lowering IOP in humans and other mammals. They are functionally defined by their ability to bind to prostaglandin-D₂ receptors of cells and evoke similar responses as when PGD₂ binds to these receptors, including the lowering of IOP. Various assays may be used for the determination of DP-agonists.

Binding assays may be used to elucidate DP-agonists of the present invention. Sharif has described a receptor binding assay in: Sharif, N.A., Williams, G.W. and DeSantis, L.M., *Neurochemistry Research*, 20:669-674 (1994), the entire contents of which are incorporated herein by reference, and may be modified for the elucidation of DP-agonists of the present invention. Briefly, the binding assays are conducted in 25 mM Tris HCl (pH 7.4) containing 138 mM NaCl, 5 mM MgCl₂, and 1 mM EDTA. Frozen-thawed expired human blood platelets (40-60 mg/ml stock) are incubated in a total volume of 500 µl with 2-10 nM [³H]PGD₂ in the absence and presence of 100 µM unlabeled PGD₂ to define total and non-specific binding, respectively. The incubations (20 minutes at 23°C) are terminated by rapid vacuum filtration, using a Whatman GF/B glass fiber filter previously soaked in 1% polyethyleneimine and 0.1% BSA, and the receptor-bound radioactivity is then determined by scintillation spectrometry. The binding data are analyzed using a non-linear, iterative curve-fitting computer program to define the receptor binding affinity (K_i) of the compounds. Compounds which exhibit K_i values in this assay of less than or equal to about 20 µM are within the definition of DP-agonists of the present invention.

The DP-agonists of the present invention are defined functionally, by their ability to stimulate adenylate cyclase activity. Sharif has described one type of functional assay in: Sharif, N.A., Xu, S. and Yanni, J.M. *Journal of Ocular Pharmacology*, 10:653-664 (1994), the entire contents of which are incorporated herein by reference and may be modified for the elucidation of DP-agonists of the present invention. Briefly, functional adenylate cyclase activity is determined using embryonic bovine tracheal cells (EbTr) cells. Cultured cells are stimulated with the test compound for 15 minutes at 23°C. The reaction is then stopped and the cAMP generated is determined by a radioimmunoassay kit. Data are analyzed using a non-linear, iterative curve-fitting computer program to define the potency ("EC₅₀", concentration which produces 50% of the maximum response of PGD₂) and efficacy of the compounds. Compounds which exhibit EC₅₀ values of less than or equal to about 10 µM are within the DP-agonist definition of the present invention.

15

Preferred DP-agonists of the present invention are of the following formula (II):



II

20

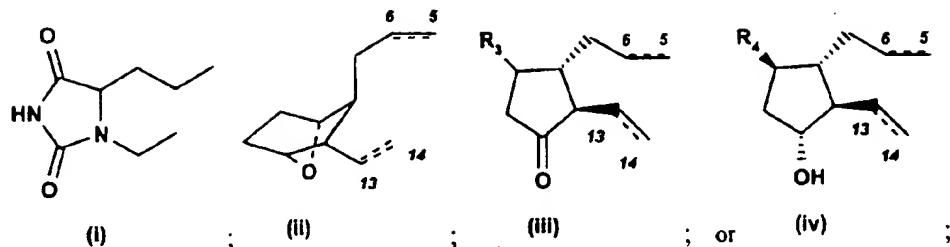
wherein:

R₁ is H, alkyl or alkylcyloalkyl;

R₂ is alkyl, cycloalkyl or alkylcycloalkyl;

25 L is carbon or oxygen; and

G is



5

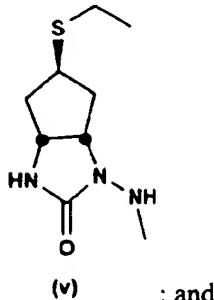
wherein:

R₃ is H, OH or alkyl;

R₄ is H, F, Cl, I, or C₁₋₃ alkyl;

— represents a single bond or double bond; provided that when a double bond is between the 13 and 14 position it is in the *trans* configuration;

when L is carbon, G may also be



15 pharmaceutically acceptable salts thereof.

Preferred DP-agonists of the present invention are those of formula (II), wherein one or more of the following substituents is selected:

20 G is (ii) and (iv);

R₄ is Cl;

R₂ is cyclohexyl;

L is oxygen;

R₁ is isopropyl; and

the bond between the 5 and 6 position is a *cis* configured double bond.

5

The most preferred DP-agonist is when G is (iv); R₄ is Cl; R₂ is cyclohexyl; L is oxygen; R₁ is isopropyl, the bond between position 13 and 14 is saturated; and the bond between the 5 and 6 position is a *cis* configured double bond.

10

Some of the DP-agonists of the present invention may be novel.

15

The DP-agonist of the present invention, aside from those compounds which may be novel, are known to those skilled in the art, and available commercially or may be made by methods known to those skilled in the art. Novel compounds of the present invention may be prepared by analogous synthetic routes as those which are known.

20

The DP-agonists wherein G is formula (i), are described in Barraclough, "Synthesis and Platelet Aggregation Inhibiting Activity of Acid Side-chain Modified Hydantoin Prostaglandin Analogues," *Archives in Pharmacology*, 326(2):85-95 (1993), the entire contents of which are incorporated herein by reference.

25

The DP-agonists wherein G is formula (ii), may be prepared from [1S-[1 α , 2 α (Z), 3 α (1E, 3S), 4 α]]-7-[3-(3-Cyclohexyl-3-hydroxy-1-propenyl)-7-oxabicyclo[2.2.1]hept-2-yl]-5-heptenoic acid following the procedure described in Das, "9,11-Epoxy-9-homoprosta-5-enoic Acid Analogues as Thromboxane A₂ Receptor Antagonists," *Journal of Medicinal Chemistry*, 33(6):1741-1748 (1990), for the conversion of [1S-[1 α , 2 α (Z), 3 α (1E, 3S, 4R)), 4 α]]-7-[3-[4-phenyl-3-(tetrahydropyran-2-yloxy)-1-pentyl]-7-oxabicyclo[2.2.1]hept-2-yl]-5-heptenoic acid

to [1S-[1 α , 2 α (Z), 3 α (1E, 3S, 4R)), 4 α]-[[4-[3-(3-hydroxy-4-phenyl-1-pentyl)-7-oxabicyclo[2.2.1]hept-2-yl]-5-heptenoic acid.

The DP-agonists wherein G is formula (iii), may be prepared from (5Z, 13E)-9S, 11R, 5 15S)-15 cyclohexyl-9-hydroxy-3-oxa-11,15-bis (tetrahydropyran-2-yloxy)-16, 17, 18, 19, 20-pentanor -5,13-prostanoic acid tert butyl ester (EP 299 914) by the method described in Bundy, "Synthesis and Platelet Aggregation Inhibiting Activity of Prostaglandin D Analogues," *Journal of Medicinal Chemistry*, 26:790-799 (1983), the entire contents of which are incorporated herein by reference.

10

The DP-agonists wherein G is formula (iv) are disclosed in WO 94/05631, and is incorporated herein by reference to the extent this publication teaches the synthesis of the compounds contained therein.

15

The DP-agonists of the present invention wherein G is (v) are disclosed in European Patent Application Publication No. 0 458 642 A1, and is incorporated herein by reference to the extent this publication teaches the synthesis of the compounds contained therein.

20

The combinations of DP-agonists and miotics according to the present invention are useful in lowering intraocular pressure and thus are useful in the treatment of persons suffering from ocular hypertension or glaucoma. As compared with therapeutically effective dosages of the individual components, the combinations produce significantly fewer unwanted side effects such as marked vasoconstriction or vasodilation of the vessels of the sclera, painful stinging, and intraocular inflammation.

25

The DP-agonists and miotics of the present invention may be formulated in various pharmaceutical compositions for simultaneous, sequential, or offset combinatorial administration to humans and other mammals as a prophylaxis or treatment of glaucoma or

ocular hypertension. For example, the DP-agonists and the miotics may be formulated together in one composition for simultaneous administration. Alternatively, they may be formulated separately for 1) concommittant dosing; 2) short delay dosing between one agent and the other; or 3) offset dosing, such as a DP-agonist dosing at night and the miotic dosed in the morning and evening.

The preferred route of administration of the compositions is topical. As used herein, the term "pharmaceutically effective amount" refers to that amount of a DP-agonist or miotic or combination of a DP-agonist and miotic which lowers IOP when administered to a mammal. The compounds of the present invention can be administered as solutions, suspensions, or emulsions (dispersions) in a suitable ophthalmic vehicle.

In forming compositions for topical administration, the DP-agonists of the present invention are generally formulated at about 0.0003 to about 0.5 percent by weight (wt%) solutions in water at a pH between 4.5 to 7.4. The compounds are preferably formulated at about 0.0003 to about 0.3 wt% and, most preferably, at about 0.003 and about 0.03 wt%. The miotics of the present invention are generally formulated at about 0.5 to 10.0 wt.%, preferably 1.0 to 3.0 wt.%. While the precise regimen is left to the discretion of the clinician, it is recommended that the resulting solutions be topically applied by placing one drop in each eye one to four times a day.

Other ingredients which may be desirable to use in the ophthalmic preparations of the present invention include preservatives, solvents, and viscosity building agents.

Preferred formulations of the present invention include the following Examples 1-3:

Example 1

Ingredient	Amount (wt%)
DP-Agonist	0.003
Miotic	1.0
Phosphate Buffered Saline	1.0
10 Polysorbate 80	0.5
Purified water	q.s. to 100%

Example 2

An example of two formulations to be used concomitantly, within 30 minutes, or offset by more than 1 hour.

5

Formulation A

	Ingredient	Amount (wt%)
10	DP-Agonist	0.001
	Monobasic sodium phosphate	0.05
	Dibasic sodium phosphate (anhydrous)	0.15
	Sodium chloride	0.75
15	Disodium EDTA (Eddetate disodium)	0.05
	Cremophor EL	0.1
	Benzalkonium chloride	0.01
	HCl and/or NaOH	pH 7.3 - 7.4
20	Purified water	q.s. to 100%

Formulation B

	Ingredient	Amount (wt%)
5	Miotic	1.0
	Monobasic sodium phosphate	0.05
	Dibasic sodium phosphate (anhydrous)	0.15
	Sodium chloride	0.75
10	Disodium EDTA (Eddetate disodium)	0.05
	Cremophor EL	0.1
	Benzalkonium chloride	0.01
	HCl and/or NaOH	pH 7.3 - 7.4
15	Purified water	q.s. to 100%

Example 3

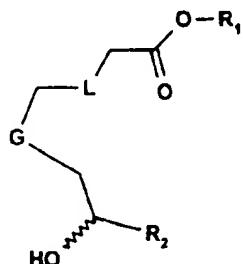
Ingredient	Amount (wt%)
DP-Agonist	0.002
Pilocarpine	0.5
Phosphate Buffered Saline	1.0
Hydroxypropyl- β -cyclodextrin	4.0
Purified water	q.s. to 100%

10

The invention has been described by reference to certain preferred embodiments; however, it should be understood that it may be embodied in other specific forms or variations thereof without departing from its spirit or essential characteristics. The 15 embodiments described above are therefore considered to be illustrative in all respects and not restrictive, the scope of the invention being indicated by the appended claims rather than by the foregoing description.

I. Claim:

1. A method for controlling intraocular pressure in a person suffering from glaucoma or ocular hypertension, which comprises administration of a DP-agonist and a miotic.
2. The method of Claim 1 wherein the DP-agonist has the following formula:

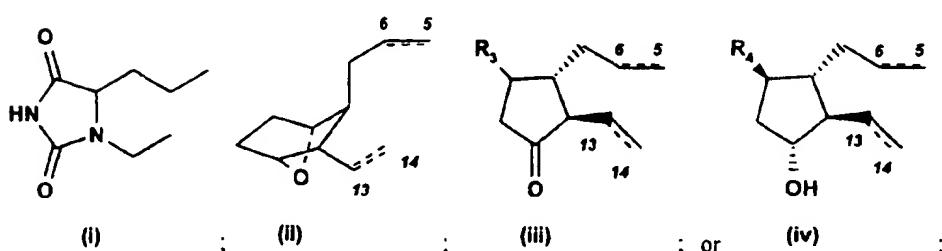


10

wherein:

- 15 R₁ is H, alkyl or alkylcycloalkyl;
 R₂ is alkyl, cycloalkyl or alkylcycloalkyl;
 L is carbon or oxygen; and
 G is

20



wherein:

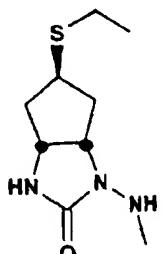
R₃ is H, OH or alkyl;

R₄ is H, F, Cl, I, or C₁₋₃ alkyl;

— represents a single bond or double bond; provided that when a double bond is between the 13 and 14 position it is in the *trans* configuration;

5

when L is carbon, G may also be



(v) ; and

pharmaceutically acceptable salts thereof.

10

3. The method of Claim 1 wherein the miotic is selected from the group consisting of pilocarpine, carbachol, and acetylcholinesterase inhibitors.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 96/18888

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K31/557 // (A61K31/557, 31:415, 31:27)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 458 642 A (WELLCOME FOUND) 27 November 1991 * see in particular claims 1-4, 14-15; page 4 lines 19-28, and page 5, lines 6-14 Y * * *	1-3
X	EP 0 458 589 A (UENO SEIYAKU OYO KENKYUJO KK) 27 November 1991 * see in particular the claims, page 3, lines 43-49, and page 7, lines 3-46 *	1-3
Y	---	1-3
	---	-/-

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

* Special categories of cited documents:

- 'A' document defining the general state of the art which is not considered to be of particular relevance
- 'E' earlier document but published on or after the international filing date
- 'L' document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- 'O' document referring to an oral disclosure, use, exhibition or other means
- 'P' document published prior to the international filing date but later than the priority date claimed

'T' later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

'X' document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

'Y' document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

'&' document member of the same patent family

1

Date of the actual completion of the international search

Date of mailing of the international search report

19 March 1997

16.04.97

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentaan 2
NL - 2280 HV Rijswijk
Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl.
Fax (+ 31-70) 340-3016

Authorized officer

Iserit, B

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 96/18888

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 94 05631 A (SCHERING AG ;BUCHMANN BERND (DE); SKUBALLA WERNER (DE); EKERDT ROL) 17 March 1994 cited in the application * see in particular page 13 (ultimate paragraph) page 14 * -----	1-3

1

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 96/18888	
---	--

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0458642 A	27-11-91	CN 1057458 A JP 7002792 A US 5147885 A	01-01-92 06-01-95 15-09-92
EP 0458589 A	27-11-91	AT 117208 T CA 2042934 A DE 69106777 D DE 69106777 T ES 2069823 T JP 2511587 B JP 4253912 A KR 9610437 B US 5397797 A	15-02-95 23-11-91 02-03-95 18-05-95 16-05-95 26-06-96 09-09-92 01-08-96 14-03-95
WO 9405631 A	17-03-94	DE 4229048 A DE 4229050 A DE 4229051 A EP 0656889 A JP 8500597 T	03-03-94 03-03-94 03-03-94 14-06-95 23-01-96

This Page Blank (uspto)